

Tattoos do not affect exercise-induced localised sweat rate or sodium concentration

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Abstract

OBJECTIVES: Skin tattoos have been shown to reduce localised sweat rate and increase sweat sodium concentration ($[Na^+]$) when sweating is artificially stimulated. This study investigated whether similar responses are observed with exercise-induced sweating. DESIGN: Unblinded, within-participant control, single trial. METHODS: Twenty-two healthy individuals (25.1 ± 4.8 y (Mean \pm SD), 14 males) with a unilateral tattoo ≥ 11.4 cm² in size, ≥ 2 months in age, and shaded $\geq 50\%$ participated in this investigation. Participants undertook 20 min of intermittent cycling (4 x 5 min intervals) on a stationary ergometer in a controlled environment ($24.6 \pm 1.1^\circ\text{C}$; $64 \pm 6\%$ RH). Resultant sweat was collected into absorbent patches applied at two pairs of contralateral skin sites (pair 1: Tattoo vs. Non-Tattoo; pair 2: Control 1 vs. Control 2 (both non-tattooed)), for determination of sweat rate and sweat $[Na^+]$. Paired samples *t*-tests were used to determine differences between contralateral sites. RESULTS: Tattoo vs. Non-Tattoo: Neither sweat rate (Mean \pm SD: 0.92 ± 0.37 vs 0.94 ± 0.43 mg \cdot cm⁻² \cdot min⁻¹, respectively; $p=.693$) nor sweat $[Na^+]$ (Median(IQR): 37(32–52) vs 37(31–45) mM \cdot L⁻¹, respectively; $p=.827$) differed. Control 1 vs. Control 2: Neither sweat rate (Mean \pm SD: 1.19 ± 0.53 vs 1.19 ± 0.53 mg \cdot cm⁻² \cdot min⁻¹, respectively; $p=.917$) nor sweat $[Na^+]$ (Median(IQR): 29(26–41) vs 31(25–43) mM \cdot L⁻¹, respectively; $p=.147$) differed. The non-significant differences for sweat rate and $[Na^+]$ between Tattoo vs. Non-Tattoo were inside the range of the within participant variability (sweat rate CVi=5.4%; sweat $[Na^+]$ CVi=4.4%). CONCLUSION: Skin tattoos do not appear to alter the rate or $[Na^+]$ of exercise-induced sweating. The influence of skin tattoos on localised sweat responses may have previously been over-estimated.

Key words: eccrine gland, thermoregulation, physical activity, fluid loss

1.0 Introduction

Sweating is a crucial thermoregulatory mechanism in humans due to its facilitation of evaporative heat loss ¹. In response to exercise-induced heat production, eccrine sweat glands can produce whole body sweat rates up to $3 \text{ L}\cdot\text{h}^{-1}$ ², with sodium concentrations ($[\text{Na}^+]$) between 15 to $120 \text{ mM}\cdot\text{L}^{-1}$ ^{3,4}. Factors that compromise the function of the sweat gland, therefore, have the potential to impair thermoregulation during exercise.

Skin tattooing involves the deposition of ink into the skin via repeated microneedle penetration and has the potential to compromise eccrine sweat gland function, and consequently thermoregulation ⁵. Given that tattoos are common, ($\sim 10\%$ of populations in some countries (e.g. France, Finland and Australia) ⁶), particularly, among physically active individuals (e.g. athletes and military personnel ⁷), it is surprising that only two published studies (~ 25 year apart) have explored the effect of tattoos on sweat responses ^{5,8}. The earliest of these, a case report ($n=1$), described a $\sim 50\%$ reduction in sweat rate responses to passive heat exposure. The recent investigation compared sweat samples taken from tattooed and non-tattooed skin of 10 participants, following electrochemical sweat gland stimulation (pilocarpine iontophoresis) using a commercial sweat collection system (Macroduct[®], Wescor, Logan, UT). Again, results indicated tattooed skin had significantly compromised sweat gland function, with $\sim 50\%$ reduced sweat rate (Non-tattooed skin = 0.35 ± 0.25 vs Tattooed skin $0.18 \pm 0.15 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$) and increased $[\text{Na}^+]$ by $\sim 35\%$ (Non-tattooed = 42.6 ± 15.2 vs Tattooed = $69.1 \pm 28.9 \text{ mM}\cdot\text{L}^{-1}$), implicating the gland's distal tubule function ⁹. These effects appeared independent of the tattoo's age (range 0.2-4 years), suggesting that skin tattooing may immediately impair regional thermoregulatory responses, and that these impairments are unlikely to resolve over time.

To date, no study has explored if skin tattoos influence exercise-induced sweating beyond the typical contralateral variation observed between two non-tattooed skin regions¹⁰. This is important, as sweat rates are considerably larger (up to $1.2 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ ³) than those

observed during artificial sweat stimulation, and the thermoregulatory load associated with exercise triggers a cascade of neural and physiological responses that determine the sweating response¹.

Therefore, the aim of this study was to explore the effect of skin tattoos on exercise-induced sweat responses. Specifically, we endeavoured to understand if the presence of a tattoo compromised exercise-induced sweat response beyond the normal variation observed between contralateral non-tattooed sites. We hypothesised that sweat samples taken from tattooed skin would demonstrate compromised function (i.e. reduced sweat rates and increased [Na⁺]) compared to contralateral non-tattooed skin samples, irrespective of the age of the tattoo, in keeping with previous reports using artificial sweat stimulation. Furthermore, we anticipated that this variation would be greater than the typical variation observed between samples collected from two contralateral non-tattooed sites within individuals.

2.0 Methods

Twenty-two healthy volunteers (14M/8F, ht = 176±9 cm, body weight = 75±13 kg) participated in this investigation involving a single laboratory visit. Individuals were eligible to participate if they met the following criteria: 1) aged between 18 and 45 years, 2) had a unilateral tattoo that was ≥2 months old, ≥11.4 cm² in size, and ≥50% shaded, and 3) deemed medically safe to undertake aerobic exercise. Table 1 describes participant and tattoo characteristics. Tattoos were categorised as ‘Dense’ if >90% of the sample area was considered shaded (*n*=14), or ‘Partial’ if shading covered 50 to 90% of the sample area (*n*=8) (based on visual inspection). All participants provided written informed consent prior to commencing the study. All data were collected in the summer months (January-March). This investigation was approved by the XXXX (removed for blinding) University Human Research Ethics Committee (Ref No. 2017/955).

Insert table 1 about here

On arrival to the laboratory ($24.6 \pm 1.1^\circ\text{C}$, $64 \pm 6\%$ RH), participants provided a urine sample for the determination of urine specific gravity (U_{SG}) and a baseline nude body weight measure to allow for subsequent determination of whole body fluid loss. If U_{SG} was ≥ 1.024 ($n=1$), indicating likely dehydration ¹¹, participants were asked to consume a bolus of water (~400 mL) prior to providing a second U_{SG} measure ~30 minutes later.

Following the hydration measures, the skin of participants was inspected to identify the most suitable sample sites. The most densely shaded tattoo site (Tattoo) with a non-tattooed contralateral (Non-tattoo) area was identified. A direct distance (Lufkin® 2m metal tape) to the nearest prominent anatomical landmark was used as a reference point to identify the appropriate contralateral site. For the control sites (Control 1 & 2), priority was given to a forearm location, ~10 cm from the wrist flexion crease, except when this area was tattooed. Once identified all sites were cleaned with ethanol, followed by distilled water, and thoroughly dried. Pilot testing of the Macroduct® sweat collection system proved unreliable in our exercise-induced sweating context. Hence, a more common exercise-sweat collection protocol was employed ¹⁰; the application of pre-weighed (HT-120, A&D Company, Japan, Precision = 0.01 g), sterile absorbent patches (Tegaderm™ +Pad (5 cm x 7 cm), 3M Deutschland GmbH, Germany, which contain an absorbance area of 2.75 cm x 4.16 cm (11.445 cm²), with a maximum capacity 1.34 g. The remaining area is comprised of a non-absorbent adhesive film). The use of absorbent patches has been shown to reflect ventilated capsule methods for measuring local sweat rates with limits of detection of ($\sim 0.12 \text{ mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$) suitable for the expected changes in exercise-induced sweating ¹². Patches at each contralateral skin site were applied simultaneously, shortly (i.e. <5 min) before commencing exercise.

To induce sweating, participants completed 4×5 min intervals (with 1 min rest between each) on an electronically braked cycle ergometer (Lode Excalibur Sport; Lode BV, Groningen, the Netherlands). The timed cycling began at a fixed power output intended to elicit a ‘hard’ rating of perceived exertion (RPE ~ 15 ¹³). Heart rate (Suunto Ambit, Finland) and RPE

were recorded at the end of each 5 min interval, at which point power output was adjusted to suit individual participant responses.

Following exercise, a short period of rest (~3-5 min) was undertaken to ensure the patches absorbed any sweat resulting from the residual heat load. Subsequently, the time from exercise commencement was recorded, and the patches were removed and weighed for determination of sweat rate using the following formula:

$$\text{Sweat rate (mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}) = \frac{(\text{Post-exercise patch (mg)} - \text{Pre-exercise patch (mg)})}{11.445 \text{ cm}^{-2}} \div \text{Collection period (min}^{-1})$$

Used patches were then placed into sterile tubes and centrifuged at 3400 rpm for 5 min to extract a sweat sample for subsequent $[\text{Na}^+]$ analysis using a calibrated sodium ion meter (LAQUA-Twin B-722, Horiba, Japan), previously validated for sweat $[\text{Na}^+]$ analysis¹⁴. All measures were performed in duplicate. Finally, participants towel dried before providing a nude body weight for the determination of whole body fluid loss.

Statistical procedures were performed using IBM SPSS, Version 25.0. All measures were examined for normality (Shapiro-Wilk test). When normally distributed, differences in mean data was analysed using parametric tests (e.g. paired-samples *t*-tests). When assumptions of normality were violated, differences were assessed using nonparametric measures (e.g. Wilcoxon signed rank test). The co-efficient of variation (CV) across the control sweat sites was considered as analytical (CV_a), within (CV_i), and between (CV_g) participant variation using traditional methods¹⁵. The CV_a of the sodium analyser has been previously determined (3.7%)¹⁶. The relationship between tattoo age and change in sweat responses between tattooed and non-tattooed skin was assessed using the correlation coefficient. All normally distributed data are presented as means and standard deviations (Mean±SD), while skewed data are presented as medians and interquartile ranges (Median(IQR)). Statistical significance was accepted as $p < 0.05$.

3.0 Results

Throughout exercise, participants reported a mean RPE of 14 ± 1.6 (average HR = 165 ± 22 bpm, av. workload = 135 ± 38 (range = 65-250) W). The exercise task resulted in a mean body weight loss of $0.55 \pm 0.33\%$ from the participant's initial nude body weight, which equated to an average sweat rate of 1.2 ± 0.6 L·h⁻¹. The mean sweat rate from contralateral control sites was not significantly different (Control 1 = 1.19 ± 0.53 vs. Control 2 = 1.19 ± 0.53 mg·cm⁻²·min⁻¹, respectively, $t(21) = -0.106$, $p = 0.917$). Similarly, the median [Na⁺] from the control sites was not significantly different (Control 1 = 29(26–41) vs. Control 2 = 31(25–43) mM·L⁻¹, $Z = -1.450$, $p = 0.147$). The CVi of participants' sweat rates and [Na⁺] across these sites was 5.4% and 3.8%, respectively. The CVg of participants' sweat rates and [Na⁺] across forearm sites only ($n = 16$) was 47% and 43%, respectively.

Participants' individual sweat rates are displayed in Figure 1. The mean sweat rate from tattooed skin was not significantly different from contralateral non-tattooed skin (Tattoo = 0.92 ± 0.37 vs. Non-tattoo = 0.94 ± 0.43 mg·cm⁻²·min⁻¹, $t(21) = -.400$, $p = 0.693$). Furthermore, when considering only densely tattooed skin ($n = 14$), sweat rates were not different from the corresponding non-tattooed skin (Dense tattoo = 0.97 ± 0.44 vs. Non-Tattoo = 0.97 ± 0.50 mg·cm⁻²·min⁻¹, $t(13) = 0.164$, $p = 0.872$).

Participant's individual sweat [Na⁺] are displayed in Figure 2. The median sweat [Na⁺] from tattooed skin was not significantly different from contralateral non-tattooed skin (Tattoo = 37(32–52) vs Non-tattoo = 37(31–45) mM·L⁻¹, $Z = -0.218$, $p = 0.827$). When considering only densely tattooed skin, sweat [Na⁺] was not significantly different from non-tattooed skin (Dense tattoo = 37(30-39) vs Non-tattoo = 36(31-39) mM·L⁻¹, $Z = -0.051$, $p = 0.959$).

No significant correlation was observed between tattoo age and percentage change in sweat rate ($r = 0.007$, $p = 0.975$) or sweat [Na⁺] ($r = -0.141$, $p = 0.532$) (Supplementary Figure).

4.0 Discussion

151 This study investigated the effect of skin tattoos on the localised sweat response during
152 exercise employing a sweat patch collection method. Contrary to our hypothesis, results from
153 the present study suggest that sweat rate and $[Na^+]$ do not differ between tattooed skin and
154 contralateral non-tattooed skin. Indeed, any variance observed between sites was within the
155 typical contralateral (non-tattooed skin) variability of individuals. Thus, tattoos are unlikely to
156 influence sweat-mediated thermoregulation in exercising individuals.

157 Two previous studies have raised concerns that skin tattoos may negatively influence
158 sweat-mediated thermoregulation ^{5,8}. The most recent (and more rigorous) of these
159 investigations employed artificial sweat gland stimulation and indicated that tattoos
160 substantially reduced sweat rate (Cohen's $d=0.79$) and increased sweat $[Na^+]$ (Cohen's
161 $d=1.01$). Given that these responses were independent of tattoo age, and were consistent across
162 all participants, the authors attributed the compromised sweat response to damage to, or
163 blockage of, the sweat glands caused by the repeated needling process involved in tattooing.
164 Indeed, fractional micro-needling radiofrequency treatment, (a procedure similar to tattooing
165 with concurrent thermal energy delivery), has recently been described as an effective treatment
166 modality for axillary osmidrosis ¹⁷. Collectively, this supports the logic of a probable
167 attenuation of sweating response in tattooed skin during exercise.

168 The current results are in contrast to our hypothesis; failing to demonstrate a tattoo-
169 mediated impaired sweating response local to the site of the tattoo. Moreover, no effect was
170 observed when the analysis was performed exclusively on the most densely shaded tattoos (i.e.
171 involving the greatest number of skin penetrations and ink deposition). The disparity in
172 observations between studies may, in part, be due to a number of methodological differences.
173 For instance, pilocarpine iontophoresis ⁵ induces sweating via local cholinergic stimulation,
174 whereas exercise-induced sweating triggers a combination of local and central mediators ¹⁸,
175 which results in considerably (~3-5 fold) higher sweat rates ³. Furthermore, the current
176 investigation employed absorbent patches to collect sweat, while a sodium ion-selective

electrode analyser was used to assess $[\text{Na}^+]$. These approaches were employed based on pilot testing and recommendations as preferred methods of sweat collection during exercise due to their accuracy, validity and practicality ^{10,12,14}. For example, the current method resulted in sweat volumes comfortably within the absorbance capacity of the patch (e.g. maximum sweat rate from any individual site = 2.65 mg/cm²/min, or 57% of the 1.34 g capacity (based on ~25 min exposure). The impact of these methodological differences was not directly investigated as the sweat collection system used in Luetkemeier et al ⁵ proved unreliable in our exercising conditions.

Results from the current investigation do not indicate a need for altered cooling, and/or nutritional (i.e. fluid administration) advice to tattooed individuals undertaking exercise. That said, it is important to acknowledge that the studies to date (including this investigation) have monitored typically small tattoos in locations not necessarily associated with anatomical regions known to have the largest sweat rates (e.g. head or back ¹⁹). Future investigations should confirm the presence of localised anatomical and/or neurological changes associated with tattooing, in particular the impact of different tattooing techniques, equipment, materials (inks), and reactions ²⁰ which may, in turn, influence sweat gland function. At present, it is unknown if large surface area tattoos, covering regions of high sweat rates, compromise skin temperature and/or whole body thermoregulatory responses to standardised thermal loads (e.g. sweating onset (via ventilated sweat capsule) or sudomotor responsiveness (via axon reflex tests).

5.0 Conclusion

Overall, the present data indicate that tattooed skin was capable of rapidly producing sweat, without influencing Na^+ resorption, in response to variable metabolic heat loads.

6.0 Practical Implications

- 201 • Previous studies have raised concerns that tattoos impair sweating responses and
202 therefore could expose individuals to greater risks of heat-related illnesses.
- 203 • Under the exercising and environmental conditions employed in this study, our data
204 suggest that skin tattoos do not appear to alter the amount (rate) or sodium concentration
205 (type) of sweat produced.
- 206 • The influence of skin tattoos on sweat responses to exercise may have previously been
207 over-estimated.

208 **Conflict of Interest:** The authors have no conflicts of interest to disclose and confirm the
209 data within is presented honestly and without fabrication, falsification, or inappropriate data
210 manipulation.

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261

262 **Figure Captions**

263 **Figure 1.** Individual sweat rates (n=22) at different skin locations. Dashed lines with open circles
264 represent partially shaded tattoos. Includes Mean \pm SD (thick solid line).

265 **Figure 2.** Individual sweat sodium concentrations [Na⁺] (n=22) at different skin locations. Dashed
266 lines with open circles represent partially shaded tattoos. Includes Median (IQR) (thick solid line).

267 **Supplementary Figure.** Correlations between tattoo age (y) and change in sweat responses
268 (%) between tattooed and non-tattooed skin (n=22).